Whole-Canopy Source-Sink Balance at Bloom Dictates Fruit Set in cv. Pinot noir Subjected to Early Leaf Removal

Tommaso Frioni,[1] Dana Acimovic,[2] Joshua VanderWeide,[3] Sergio Tombesi,[1] Alberto Palliotti,[4] Matteo Gatti,[1] Stefano Poni,[1] and Paolo Sabbatini[3]*

Abstract: Severe prebloom leaf removal dramatically affects the source-sink balance in grapevines, leading to a reduction in fruit set. In this study, carried on for two consecutive years, the impact of defoliation at bloom was evaluated with the objective to assess the capacity of developing inflorescences to attract photosynthates from adjacent shoots subjected to varying source/sink manipulations. In Pinot noir trained to a bilateral cordon, untreated vines (UT-UT) were compared to a treatment where shoots on one-half of the vines were subjected to the removal of 10 basal leaves at bloom (UT-LR). Another set of vines underwent sink removal (inflorescences and shoot apex) at bloom on half of the shoots by hand thinning (TFR-UT). A final treatment consisted of removing 10 basal leaves on half of the shoots and removing sinks on the other half of the canopy (TFR-LR). Following treatment application, shoot leaf area retained was ~40% of the total in UT-LR and TFR-LR. UT-LR reduced the whole canopy leaf area available per inflorescence by ~44% when compared to UT-UT. TFR-LR did not affect this balance. In UT-LR, fruit set was significantly reduced (-36% as compared to UT-UT), whereas it was unaffected by TFR-LR and TFR-UT. Independent of treatments, fruit set in both seasons was correlated with whole-canopy leaf area per inflorescence at bloom and not with the single shoot leaf area retained after early leaf removal.

Key words: fruit set, leaf removal, reproductive activity, translocation

In grapevine, nutrition of flower ovaries during bloom and fruit set is conditioned by two main carbon sources. The first is the carbohydrates stored in permanent wood in the form of starch and then mobilized during early spring shoot growth (Zapata et al. 2004); the second is the photosynthates produced by leaves immediately after budbreak as they reach their mature stage (Lebon et al. 2008). During berry growth and through ripening, developing clusters attract assimilates from their shoot of origin and from adjacent shoots, roots, and permanent organs (Mansfield and Howell 1981, Hunter and Visser 1988, Candolfi-Vasconcelos et al. 1994). On the contrary, earlier in the season, inflorescences are a weaker sink and their ability to attract nutrients is considerably reduced, making them more sensitive to low source availability than vegetative organs (Hale and Weaver 1962, Candolfi-Vascon-celos and Koblet 1990, Frioni et al. 2018). This is because grapevine inflorescences develop during a time when rapidly growing shoot meristems and young leaves are stronger sinks (Keller 2015, Frioni et al. 2018), making them better competitors in terms of acquiring carbon from source tissues (Obeso 2002, Frioni et al. 2018). Inadequate availability of carbohydrates compromises embryo development, with consequent abortion resulting in a reduced fruit set (Lebon et al. 2008).

Grapevine interactions between sources and sinks can be exploited in vineyard management when cluster compactness is an issue for grape sanitation and quality at harvest. It is well-understood that the source limitation, induced by early leaf removal applied around bloom, causes a reduction in fruit set (Poni et al. 2008, Acimovic et al. 2016). The explanation for this effect often reported in the literature is related to cluster carbon starvation caused by the drastic removal of the most photosynthetically active portion of the shoot at an early stage of the grapevine growth and development (Poni et al. 2008, Palliotti et al. 2011). In fact, removing most of the photosynthetically active leaf area around bloom reduced fruit set by ~20 to 40%, depending on cultivars and viticultural areas, lowering cluster compactness and improving fruit technological maturity and polyphenolic composition at harvest (Bennett et al. 2005, Poni et al. 2005, Gatti et al. 2012, VanderWeide et al. 2018). In particular, the effects of early leaf removal at bloom on fruit set are inversely correlated to the amount of leaf area (LA) retained (Acimovic et al. 2016). For instance, in Michigan (USA), the removal of six to eight basal leaves was identified as the minimum threshold to reduce fruit set and, in turn, cluster compactness in Pinot noir grapevines, whereas the removal of less than six leaves was not enough to produce the same effects (Acimovic et al. 2016). However, early in the season, vegetative growth is often not uniform

within the vine. Shoot length and LA can be highly variable among shoots on the same vine, especially in cane-pruned training systems (Keller 2015, Gatti et al. 2018). Consequently, by removing a certain number of leaves, the LA retained on each shoot can differ greatly based on shoot-specific vigor. Moreover, large-scale applications of early leaf removal are often executed by pneumatic machines in cool-climate viticultural regions, blowing air at high pressure and shredding leaves (Hed and Centinari 2018, VanderWeide et al. 2018), and the LA retained on different shoots might change based upon their length and canopy position. This creates the possibility for distinct scenarios as a consequence of variance in leaf removal, depending on the ability of inflorescences to attract photosynthates from other shoots. Under the best-case scenario, if carbohydrates are cross-translocated among shoots, then the fruit-set reduction should not substantially vary between different vine shoots; otherwise, changes in fruit set should be sensibly variable on a shoot basis. A second scenario, taking into account that the correlation between the number of leaves removed and the fruit-set intensity is not linear (Acimovic et al. 2016, Frioni et al. 2018), the beneficial effect of leaf removal could be limited to a few clusters with a possible increase of nondesired intravine variability of grape ripening and quality. However, translocation of photosynthates between different shoots during berry growth and ripening is already known (Mansfield and Howell 1981, Hunter and Visser 1988), but the contribution of assimilates produced on distant shoots to developing inflorescences at bloom and fruit set has not previously been explored in the literature. This may play a pivotal role in setting the fruit-set rate in a specific production system, such as that used for raisins, in which there is a strong yield imbalance between canes on the same vine or in trellis systems (e.g., Scott Henry, Smart Dyson, bilateral cordons, Geneva Double Curtain or GDC) in which multiple cordons of different vigor can lead to very variable cane source-sink ratio on the same vine. The impact of this variability on yield and quality can be emphasized in specific phenological phases in which carbohydrate availability is particularly limiting, such as through flowering and fruit set.

Our hypothesis is that inflorescences with extremely low source availability are able to attract photosynthates from distant shoots in a source-sink-dependent manner, affecting fruit set and suggesting its stronger relationship with whole-canopy source-sink balance at bloom than with single shoot retained LA. Therefore, the aim of this work was to clarify whether the effects of early defoliation at bloom on fruit set for a given cluster is primarily related to the LA retained on a single shoot, or if the total vine LA availability contributes to this process. We compared vines subjected to the removal of 10 basal leaves at bloom on half of their shoots with untreated vines and vines on which leaf removal was performed on half of the shoots and coupled with remaining shoots subjected to cluster removal.

Materials and Methods

Plant material and experimental design. Twenty-yr-old *Vitis vinifera* L. vines cv. Pinot noir Mariafeld clone (FPS29) grafted on 3309C rootstock were used in this study during the 2011 and 2012 growing seasons. The vineyard was located at the Southwest Michigan Research and Extension Center (40°09′N; 86°36′W; 220 m asl) near Benton Harbor, Michigan. Vines were planted in Spinks loamy fine soil, with a north-south orientation and a spacing of 1.8 m between vines and 3.0 m between rows, and trained to a vertical shoot positioning system with two bilateral spur-pruned cordons (Figure 1). Vines were spur-pruned during the winter, leaving ~60 buds/vine. Around 3 wks before bloom, the number of inflorescences was adjusted to be equal on each side of the
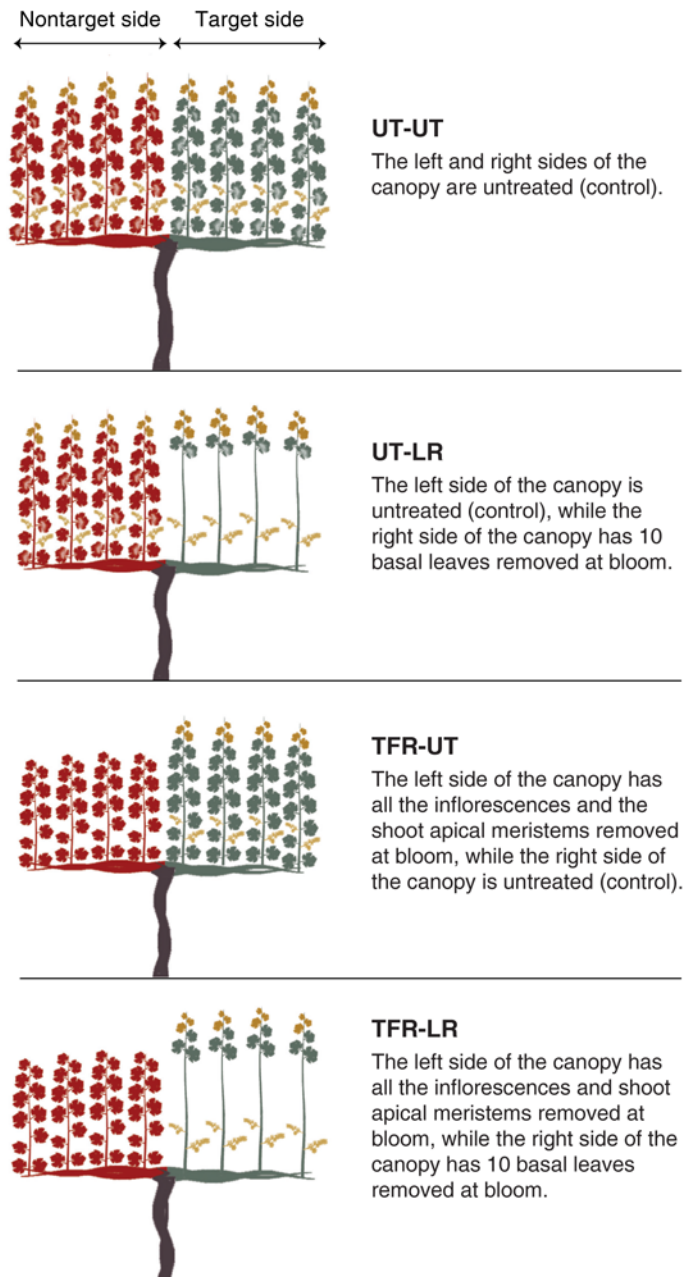

Figure 1 Diagrammatic representation and description of experimental treatments applied at bloom in 2011 and 2012. UT: untreated; LR: leaf removal; TFR: Tips and flowers removal. The cordon on the right (target side) was used for detailed data collection during vine growth and development over the two experimental seasons.

bilateral cordon (~50/side). Recommended crop protection practices were followed, and the pest management program was based on scouting, experience, and weather conditions. During bloom, to avoid potential mechanical damage to flowers by the sprayer, no application of fungicide or insecticide was performed for 2 wks. Relevant meteorological data were recorded during the experiment by an automated weather station from the Michigan Automated Weather Network located on the site at 120 m from the experimental vineyard. Total monthly precipitation; daily precipitation; daily minimum, maximum, and average temperature; and growing degree days (GDD) were calculated with the Baskerville-Emin method using a base temperature of 10°C (Baskerville and Emin 1969). No irrigation was used and standard summer vineyard practices were applied. Vines were manually hedged on 25 July, day of year (DOY) 206, in both 2011 and 2012. The main phenological stages were identified using Eichhorn and Lorenz (E-L) (1977) and recorded in both 2011 and 2012.

The experiment was arranged as a randomized complete block design with two factors (treatment and year). A section of the 2.5-ha vineyard composed of 64 vines was divided into four blocks of 16 vines each in a contiguous vine row with guard vines at the beginning and the end of each block. Within each block (random effect), the bilateral cordon vines were divided into the following treatments (Figure 1): untreated vines on both sides of the bilateral cordon (UT-UT); untreated on the non-target side of the cordon but subjected to early leaf removal (10 basal leaves per shoot removed) on the target side (UT-LR); subjected to the removal of inflorescences and shoot tips at bloom (10% open flowers) on the non-target side but untreated on target side (TFR-UT); and subjected to the removal of inflorescences and shoot tips at bloom on the non-target side and subjected to early leaf removal (10 basal leaves) on the target side (TFR-LR).

Early leaf removal of the south side of the bilateral cordon (LR hereafter) involved removing 10 basal leaves from shoots at bloom, identified as a threshold able to induce a significant reduction of fruit set (on a whole-canopy basis) in Pinot noir grown in Michigan. The manual removal of shoot tips and inflorescences on the north side of the bilateral cordon (tip and flower removal, TFR hereafter) was executed at bloom by removing all the already developed inflorescences with scissors and hedging the apical part (~5 cm) of shoot tips. A vine per treatment in the middle of each block was tagged for detailed measurements of shoot growth and LA development. Additionally, three shoots from each tagged vine were randomly chosen on the south side (target side hereafter) of the canopy and tagged for the entire duration of the experiment. Detailed measurements of shoot length, degree of fruit set, and cluster parameters were performed on the target side of the vine (south side) to evaluate the impact of the treatments performed on the north side of the canopy (Figure 1). The target side of the bilateral cordon induced different levels of source reduction, while the nontarget side of the canopy promoted varying levels of sink reduction (Figure 1).

Estimation of LA. Shoot LA was estimated after Acimovic et al. (2016). Shoot length was measured weekly over a period of ~40 days, starting from 7 June in 2011 and from 3 June in 2012 and ending on 13 July in both years, which corresponded to ~1 wk before bloom to 1 mo after bloom, and then shoot growth rate was calculated. A sample of 20 shoots, collected weekly from guard vines (subjected to similar canopy management of UT-UT), was used for estimation of the total LA per shoot (main shoot LA). In the laboratory, shoot length was measured and shoot LA was determined by measuring the single LA with an LA meter (LI-3050AHS, LI-COR Biosciences). A linear relationship between LA (y) and shoot length (x):

$$y = 16.29x - 277.69, R^2 = 0.84 \text{ (for 2011) and}$$
$$y = 16.26x - 94.59, R^2 = 0.88 \text{ (for 2012)}$$

was used for estimation of total LA. After the application of the treatments (LR and TFR), leaves removed per shoot were collected in Ziploc (SC Johnson) bags and LA removed on each shoot was quantified with the LA meter. LA removed was then subtracted from total LA for calculation of retained LA. At the time of treatment application (bloom), the number of shoots per vine was counted and whole-canopy LA at bloom was calculated by multiplying the average LA retained on shoots subjected to LR, TFR, or UT, with the number of shoots of each type present in the different vines.

Fruit set estimation, harvest parameters, and cluster morphology. Fruit set was estimated on the target side of the bilateral cordon after Poni et al. (2006). At developmental stage E-L 20 (onset of bloom), the three basal inflorescences arising from the tagged shoots of each vine were photographed against a dark background. At the same time, samples of 20 inflorescences at developmental stage E-L 20 from the guard vines were also photographed against a dark background and then separately collected in Ziploc (SC Johnson) bags and transported to the laboratory. The actual number of flowers composing the collected inflorescences was destructively counted, and the number of flowers visible in the photos for the same inflorescences was recorded. A linear regression between the actual number of flowers (y) and the flowers counted in pictures was built:

$$y = 1.81x, R^2 = 0.90 \text{ for 2011 and}$$
$$y = 1.49x, R^2 = 0.87 \text{ for 2012.}$$

Then, the number of flowers for inflorescences photographed in the field from tagged shoots was counted and recorded. The linear correlations were used to estimate the number of flowers of each tagged shoot basal inflorescence. Fruit set (%) was calculated based on the number of flowers estimated at bloom for each cluster and the respective number of berries counted at harvest.

Harvest was set as the achievement of the optimal maturity threshold fixed as ~22 Brix. All experimental vines were harvested the same day and the number of clusters per cordon side was recorded. The three basal clusters on the three tagged shoots of each vine were sampled and brought to the laboratory. The cluster weight was determined, then the number of berries per cluster was counted, and the main rachis length was measured. The average berry weight was then obtained. The cluster compactness index (CCI) was

calculated by dividing the number of berries per cluster by the rachis length.

Statistical analysis. The experiment was analyzed with randomized block designs with two factors (treatment and year). The experimental planting consisted of 64 vines and was divided into four blocks of 16 vines each. Data were analyzed using two-way analysis of variance (treatment, year) in the PROC MIXED procedure, SAS 9.3. Means were separated by the Student-Newman-Keuls test. Only the evolution of shoot growth and LA was analyzed separately for each year to assess the impact of climatic conditions on those variables. Regression analysis was performed using Sigma Plot 11 (Systat Software, Inc.). R^2 statistical significance was tested by t-test as described (Sokal and Rohlf 1969).

Results

Weather conditions. Weather conditions during the two experimental years were similar. In particular, when compared with the historical mean of the experimental site (1560 GDD), 2011 had a slightly lower heat accumulation from 1 April to 31 Oct (1467 GDD), while 2012 was above the average (1635 GDD). Overall, analogous daily temperatures and seasonal heat accumulation in the two seasons were reported the week before and after the treatment application (Figure 2). In particular, no extreme temperature or rain events were recorded at the time of bloom and fruit set, and comparable GDD accumulated early in the two seasons (Figure 2), allowing us to assess the reproducibility of the results in both years.
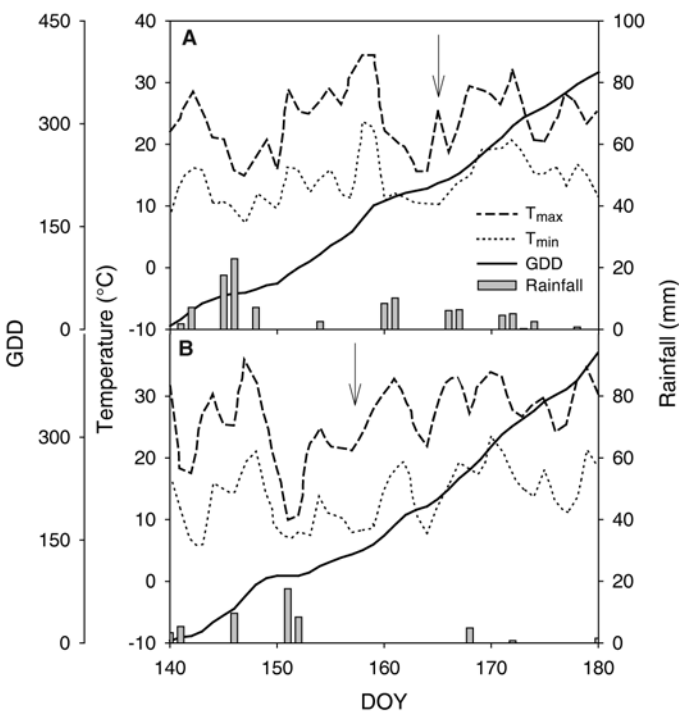
LA, canopy growth, and vine balance at bloom. Before bloom, no difference between treatments was found in shoot growth rates in either experimental season, 2011 or 2012 (Figure 3). In 2011, after the LR treatment, TFR-UT had a significantly faster shoot growth rate than other treatments from bloom until DOY 175. On the contrary, UT-LR had a short-term decreasing growth rate before increasing on DOY 165. On DOY 172, a significant difference was still present between UT-LR and TFR-UT, whereas later in the season, no differences between treatments were found. After the implementation of treatments in 2012, shoot growth rate was significantly reduced in UT-LR until DOY 170, whereas no differences were found between other treatments or later during vine growth. The evolution of LA followed a similar pattern in both 2011 and 2012 (Figure 4). While initially LA was not different between treatments, application of defoliation led to reduced shoot LA (main leaves on target side of the bilateral cordon) in LR treatments, and the difference remained during the duration of the experiment.

After treatment application, the retained shoot LA (target side) of UT-LR was 24% of UT-UT (Table 1). No differences in LA were found between TFR-UT and UT-UT, or between UT-LR and TFR-LR. On a whole-canopy basis, ~1.62 m²/vine were removed from each LR treatment. After treatment application, UT-LR had a significantly lower vine LA calculated at bloom (LA_{bloom}) when compared to UT-UT. Similarly, TFR-LR had a vine LA_{bloom} comparable to that

Figure 2 Daily precipitation (bars) and minimum (dotted line), maximum (short dash line), and growing degree days (GDD, solid line) during flowering and pea-size berry stage in 2011 (**A**) and 2012 (**B**) at Southwest Michigan Research and Extension Center (Michigan). Arrows indicate the time of treatment application; DOY: day of year.

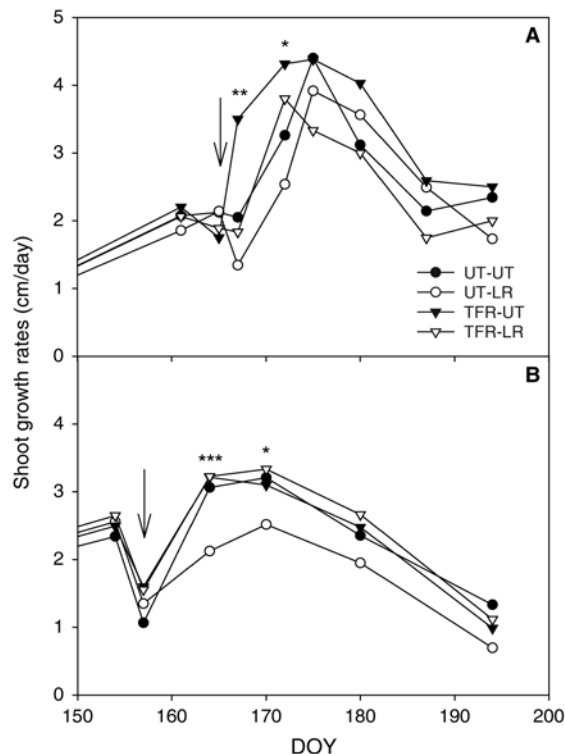**Figure 3** Daily growth rates of shoots located on the right target side of the canopy in vines subjected to an artificial source/sink balance modification at bloom in 2011 (**A**) and 2012 (**B**). Arrows represent the time of treatment application. *, **, and *** indicate significant differences at $p \leq 0.05$, 0.01, and 0.001, respectively. UT: untreated; LR: leaf removal; TFR: Tips and flowers removal; DOY: day of year.

measured in UT-LR, whereas in TFR-UT, it was comparable to UT-UT. Treatments affected significantly the vine balance at bloom, indexed as vine LA per number of inflorescences. While TFR-LR had a vine LA_{bloom}/inflorescence similar to the one recorded in UT-UT vines, leaf removal reduced vine LA_{bloom} available per each inflorescence in UT-LR by 44% when compared with UT-UT. In contrast, TFR-UT increased the ratio by 97% when again compared to UT-UT (Table 1).

Impact on fruit set. The treatment applications significantly impacted fruit set (Table 2). UT-LR reduced fruit set by 33% compared to UT-UT; meanwhile, TFR-LR and TFR-UT did not affect the number of flowers setting berries. When compared to the reduced amounts of LA retained in shoots located in the target side of the bilateral cordon, the TFR-LR treatment showed higher fruit set when compared to UT-LR in both years (Table 2), even if the amount of removed LA was similar (Table 1). Interestingly, fruit set was not related to the shoot-retained LA (Figure 5). Instead, fruit set was significantly correlated to the ratio between whole canopy LA_{bloom} and number of inflorescences, regardless of the treatment (Figure 6). In both years, the equation fitting the data set was an exponential rise to maximum:

2011: $y = 44.1*(1-e^{-0.004x})$, $R^2 = 0.84$, $P = 0.04$;
2012: $y = 39.1*(1-e^{-0.006x})$, $R^2 = 0.90$, $P = 0.02$.

Harvest parameters and cluster morphology. TFR treatment impacted clusters per vine and yield (Table 2), with both

producing an average of ~39% and ~36% less than UT-UT, respectively. In contrast, UT-LR did not affect productivity or cluster number when compared to TFR-LR. UT-LR had a significantly lower berry number per cluster (-29%), CCI (-26%), and cluster weight (-16%) when compared to UT-UT, whereas TFR-UT and TFR-LR were not different from the untreated vines. Finally, rachis length and berry size were not impacted by treatments. In general, berries per cluster and vine productivity were higher in 2011 than 2012.

Discussion

The strategy of this research utilized the target side (south) of the bilateral cordon as a way to induce different means of a source reduction and the nontarget side (north) of the canopy to promote varying levels of sink reduction (Figure 1). In target shoots of the south side of the canopy, LR treatments caused an abrupt and severe decrease of source availability, whereas TFR treatments ameliorated this effect in nontarget shoots in the north part of the canopy that removed all of the active sinks (apex and clusters, Figure 1). This led to treatments where the individual shoot source/sink balance was skewed, but the whole canopy balance was not. This was reflected in treatments that changed the single shoot and whole canopy LA_{bloom}/inflorescence ratio in both years (Table 1, Figures 5 and 6). For instance, TFR-LR likely caused the largest source/sink imbalance between the two cordons, yet source/sink ratio was unaffected, taking into account the whole canopy, as the removal of leaves on one side of the bilateral cordon was counterbalanced by the removal of inflorescences on the other side (Figure 1). This resulted in a vine LA_{bloom}/inflorescence ratio that was not different from UT-UT (Table 1). On the contrary, UT-LR reduced the vine LA_{bloom}/inflorescence ratio of both the shoot of the south side of the
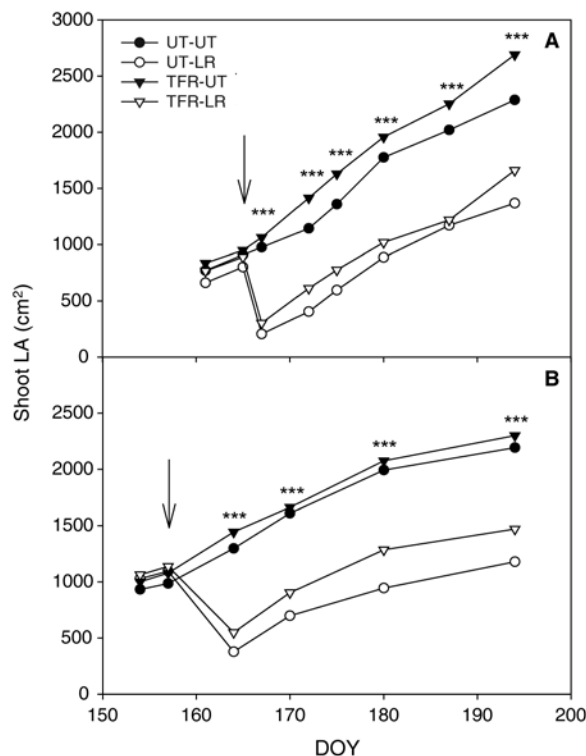

Figure 4 Evolution in the leaf area (LA) of main shoots located on the right target side of the canopy in vines subjected to an artificial source/sink balance modification at bloom in 2011 (**A**) and 2012 (**B**). Arrows represent the time of treatment application. *** indicates significant differences at $p \leq 0.001$. UT: untreated; LR: leaf removal; TFR: tips and flowers removal; DOY: day of year.

Table 1 Amount of leaf area (LA) removed by the different treatments in Pinot noir grapevines grown at the Southwest Michigan Research and Extension Center (Michigan) expressed on a shoot and a whole vine basis and calculation of the vine source/sink balance at bloom.

Treatment (T)[a]	LA				LA_{bloom}/ inflorescence[c] (cm²)
	Removed[b] (cm²/shoot)	Retained[b] (cm²/shoot)	Removed[c] (m²/vine)	Retained[c] (m²/vine)	
UT-UT	0 b[d]	867 a	0 b	3.54 a	397 b
UT-LR	723 a	210 b	1.49 a	2.26 b	223 c
TFR-UT	0 b	1032 a	0 b	3.86 a	782 a
TFR-LR	900 a	160 b	1.75 a	2.17 b	365 b
Year (Y)					
2011	323	539	0.63	2.90	371
2012	466	590	0.91	3.00	494
T	***[e]	***	***	***	***
Y	ns[e]	ns	ns	ns	ns
T × Y	ns	ns	ns	ns	ns

[a]UT: untreated; LR: leaf removal; TFR: tips and flowers removal.
[b]Parameters assessed only in the targeted side of the canopy.
[c]Parameters assessed on whole-vine basis. LA_{bloom}: LA calculated at bloom.
[d]Means within columns noted by different letter are different by Student-Newman-Keuls test ($P = 0.05$).
[e]*** and ns indicate significant differences at $p \leq 0.001$ or not significant, respectively.

canopy (target) and the whole canopy because of less available assimilates from the north side of the canopy (nontarget). Despite unaffected target shoot LA, TFR-UT increased this ratio due to altered vine source/sink balances (Table 1).

The source/sink indexes usually adopted to define vine balance are static and are typically used to describe later stages of development, such as veraison or harvest (Kliewer and Dokoozlian 2005). The definition of vine balance at bloom instead should take into account the main canopy source and sink organs present around bloom (Lakso and Sacks 2009, Poni and Bernizzoni 2010). However, estimating source/sink balance at this time is complicated by the transition of vines from vegetative to reproductive growth, when the pool of starch stored during dormancy is almost depleted (Zapata et al. 2004) due to reserves being mobilized to support vegetative flush (Zimmerman 1971, Scholefield et al. 1978, Zapata et al. 2004). As such, treatments (LR, TFR) perturbing this delicate balance can severely affect the carbohydrate availability, increasing the importance of the new assimilate photosynthates in feeding physiological activities in comparison with reserves in woody tissues (Candolfi-Vasconcelos et al. 1994, Caspari et al. 1998, Zapata et al. 2004, Lebon et al. 2008, Frioni et al. 2018). For these reasons, vine LA_{bloom} per each inflorescence can be used as a ratio to determine potential alteration of the vine (or the shoot) source/sink balance.

UT-LR caused a transient depression in shoot growth (Figure 3) and a reduction of LA until hedging in both years (Figure 4). This is consistent with Hunter and Visser (1990), who suggested that early defoliation can reduce the main shoot length and promote lateral shoot growth. Interestingly, this was not the case of TFR-LR, where the expected limitation of LA did not correspond to a shoot growth reduction. Shoot apexes are strong competitors in terms of carbon demand around bloom, and their relative sink strength increases when the pool of carbohydrates is reduced (Obeso 2002, Frioni et al. 2018). In fact, the photosynthetic activity of retained leaves is more efficient due to a decrease of the sugars' feedback inhibition because of the concomitant increase in the apex sink strength and the low source availability (Lemoine et al. 2013). The physiological effect is related to the higher carboxylation efficiency, as well as an enhanced capacity for regeneration of ribulose-1.5-bisphosphate (Flore and Lakso 1989). Overall, in our experiment, LR induced a severe reduction of carbon availability that affected, even if for a short period, the shoot growth rates; however, this did not occur when part of the canopy was subjected to the removal of the carbon sinks (clusters and shoot tips). On the other hand, our experiment points out that the loss of a source counterbalanced by a concurrent loss of a sink on another part of the canopy does not cause a decrease of vegetative growth. Furthermore, focusing on the reproductive activity, the LR counterbalanced by the concurrent removal of cluster and shoot tips in another part of the canopy contributed to preserve fruit set at levels compatible with that of the UT-UT and TFR-UT. These results suggest that, in grapevine, the manipulation of the source/sink balance on one cordon can counterbalance the source deficit in another cordon in a part of the canopy not directly connected via vascular system between leaves and root system. The observed effect on fruit set, a relatively short phenological phase, indicates a fast reorganization of carbon partitioning that is able to re-equilibrate the source sink balance in a few days. These results point out a contrasting behavior to tree species, where large to small organs (i.e., branch to spur) are semi-autonomous for carbon budget (Marsal et al. 2003, Lampinen et al. 2011, Tombesi et al. 2015, Reyes et al. 2016). In species such as almond, apple, and peach, organ-selective sink (fruit) or source (leaves) removal or variability affected only the source/sink balance on the same organ and not that of a nearby organ. This is not the case with grapevine, which, according to our experiment, shows a low degree of semi-autonomy with a fast redistribution of exceeding carbohydrates.

Early leaf removal is a widely adopted viticultural practice to reduce fruit set, lower cluster compactness, and improve

Table 2 Vine productivity, fruit set, and cluster morphology in Pinot noir grapevines grown at the Southwest Michigan Research and Extension Center (Michigan).

Treatment (T)[a]	Fruit set[b] (%)	Berries/ cluster[b] (n)	Berry wt[b] (g)	Rachis length[b] (cm)	CCI[b]	Cluster wt[b] (g)	Clusters/ vine[c] (n)	Yield[c] (kg)
UT-UT	36.5 a[d]	98 a	0.82	12.0	8.04 a	82.5 a	95 a	7.98 a
UT-LR	24.6 b	70 b	0.94	12.7	5.95 b	68.1 b	106 a	7.36 a
TFR-UT	38.3 a	103 a	0.86	12.2	8.86 a	91.3 a	52 b	4.55 b
TFR-LR	32.4 a	99 a	0.85	14.6	8.36 a	86.1 a	63 b	5.70 b
Year (Y)								
2011	31.4 b	103 a	0.84	14.9 a	8.40	88.7 a	88	7.63 a
2012	34.4 a	83 b	0.91	11.4 b	7.42	77.0 b	72	5.47 b
T	***[e]	***	ns	ns	***	***	***	***
Y	*	***	ns	**	ns	*	ns	***
T x Y	ns	ns	ns	ns	ns	ns	ns	ns

[a]UT: untreated; LR: leaf removal; TFR: tips and flowers removal.
[b]Parameters assessed only in the targeted side of the canopy. CCI = cluster compactness index (g of fruit per cm of rachis).
[c]Parameters assessed on whole-vine basis.
[d]Means within columns noted by different letter are different by Student-Newman-Keuls test (P = 0.05).
[e]*, **, ***, and ns indicate significant differences at $p \leq 0.05$, 0.01, 0.001, or not significant, respectively.

vineyard efficiency and fruit composition at harvest (Poni et al. 2005, 2006, Palliotti et al. 2011, Gatti et al. 2012, Vander-Weide et al. 2018). Acimovic et al. (2016) found that the removal of six to eight basal leaves on all shoots of the vine was the optimal threshold to reduce fruit set, lower cluster compactness, and improve fruit composition in Pinot noir grown in Michigan. The removal of 10 basal leaves at bloom in target shoots induced severe carbohydrate stress, which reduced fruit set by 44% and cluster weight and yield by 50% (Acimovic et al. 2016). In our trial, conducted under the same viticultural conditions, UT-LR decreased fruit set and cluster weight by 33% and 17%, respectively, but did not affect yield due to an unbalanced number of clusters per vine and a higher photosynthetic efficiency of the younger leaves grown after the leaf removal treatment during the entire fruit-ripening period (Palliotti et al. 2011). In TFR-LR, where vines received the same LR treatment, fruit set and cluster weight were not changed from UT-UT. Shoot LA was similar on nontarget shoots between LR treatments, suggesting that the whole canopy LA_{bloom} has a greater impact on fruit set than the single shoot LA_{bloom}. Therefore, we expected to observe a higher impact on fruit set of the target side of UT-LR canopies and a consequent reduction of cluster weight in line with data reported by Acimovic et al. (2016). However, we also intended to test if LA_{bloom} retained on the single shoot was the driving force affecting fruit set, leading in turn to a comparable fruit set in the target side of TFR-

LR and UT-LR treatments. Conversely, TFR-LR had a fruit set comparable to UT-UT. This suggests that in TFR-LR, the TFR treatment performed on the nontarget side of the bilateral cordon affected the fruit set of the target side of the bilateral cordon subjected to LR. Interestingly, TFR-UT did not increase fruit set. Caspari et al. (1998) increased fruit set by girdling the base of shoots, stating that extra supplies of carbohydrates produced by leaves can increase the fruit set of inflorescences borne by the same shoot, if this is isolated from the parental vine. However, regardless of the inflorescences' carbon demand and position, fruit set has a seasonal maximum threshold defined by genotype and environmental conditions (May 2004), and the lack of difference between UT-UT and TFR-UT might be related to the achievement of this threshold under UT-UT conditions.

Interestingly, no correlation was found between shoot LA_{bloom} and fruit set. These results are due to the difference in fruit set of the target shoot in UT-LR and TFR-LR, despite a similar amount of target shoot LA_{bloom} (Figure 5). On the contrary, independent of the treatment, correlations were identified for whole canopy LA_{bloom}/inflorescences and fruit set in both years (Figure 6). The equation plateaued in both years at a level of fruit set that can be considered the seasonal genotype × environment maximum value (May 2004), or at least a value that cannot be further augmented by marginal increases of the vine LA_{bloom}/inflorescences ratio. In fact, all vines having an LA_{bloom}/inflorescences ratio >400 cm²/
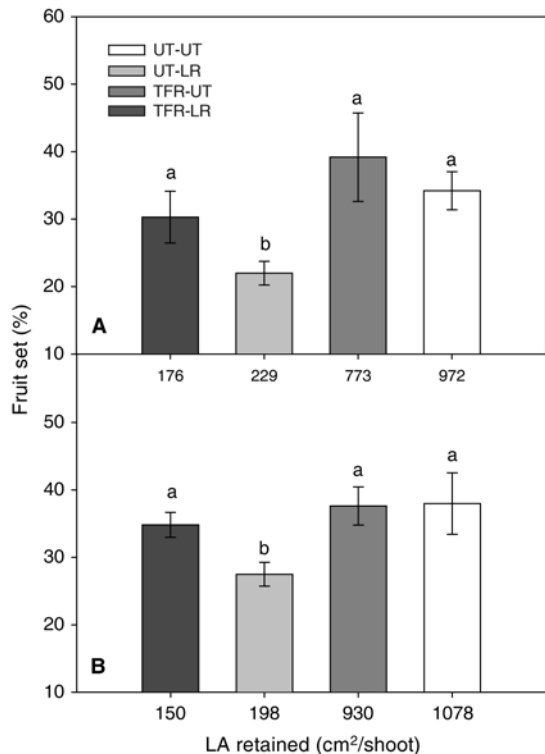

Figure 5 Fruit set of clusters in the shoots located on the right target side of the vine canopy, having different leaf area (LA) at bloom as a result of an artificial source/sink balance modification in 2011 (**A**) and 2012 (**B**). Each bar represents the means of 12 values ± SE. Means noted by different letters are different by Student-Newman-Keuls test ($P = 0.05$). UT: untreated; LR: leaf removal; TFR: tips and flowers removal.
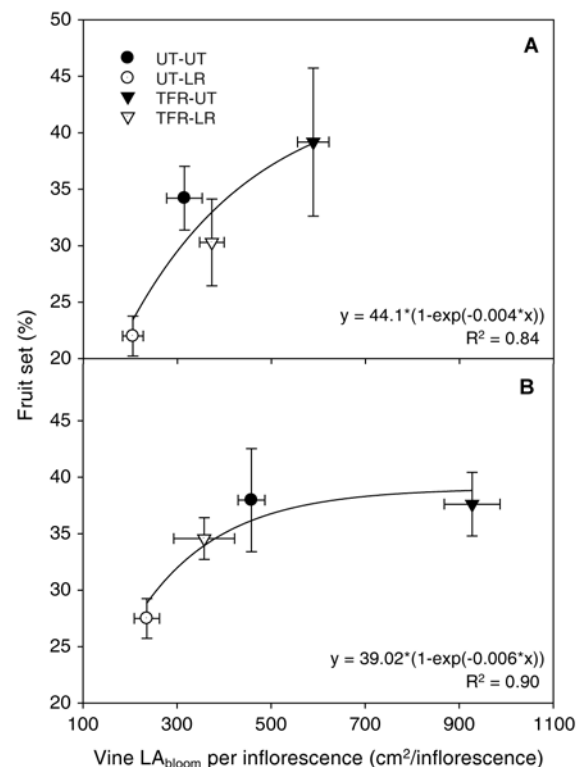

Figure 6 Regression between the vine balance at bloom and fruit set in vines subjected to an artificial source/sink ratio modification at bloom in two canopy sections in 2011 (**A**) and 2012 (**B**). Means ± SE (n = 12). UT: untreated; LR: leaf removal; TFR: tips and flowers removal; LA: leaf area; LA_{bloom}: LA calculated at bloom.

inflorescence had a similar fruit set. TFR-LR vines, despite lower loads of inflorescences, had a vine balance similar to UT-UT and a fruit set similar to UT-UT and TFR-UT. This behavior suggests a scenario in which the higher availability of carbon on a proportion of shoots with an excess supply of photoassimilates is translocated to inflorescences located on other shoots in condition of source limitation to satisfy their higher carbon demand. Although the translocation of photosynthates between close shoots at later developmental stages such as berry development and ripening was already established (Mansfield and Howell 1981, Hunter and Visser 1988, Intrigliolo et al. 2009), our data suggest a high capability of carbon redistribution at bloom, even between different cordons. However, Quinlan and Weaver (1970) found that labeled carbon was not translocated between shoots at bloom. Instead, carbohydrates were translocated from a shoot to an adjacent one if the latter one was defoliated or if the first one was hedged and deflowered (Quinlan and Weaver 1970). Our results demonstrate that translocation of photosynthates at bloom occurs in response to timing and intensity of leaf removal, and that they contribute to satisfy the carbon demand of inflorescences located on other shoots. However, carbohydrates are translocated from other shoots only in the presence of a carbon deficiency or if their translocation does not contribute to the development of nondemanding inflorescences, which is in accordance with Quinlan and Weaver (1970). Consequently, the effects of early leaf removal on fruit set and cluster compactness depend on the whole canopy source/sink balance at bloom and not on availability of LA_{bloom} within the same shoot. This can be translated into field viticultural practices by suggesting that any shoot hedging should be done quite far, in time, from the application of the leaf removal, potentially offsetting the impact on fruit set.

Conclusions

In grapevine, fruit set occurs during a stage when carbon availability is critical, and changes of source availability can significantly affect fruit set and cluster morphology at harvest. However, vines can count on the translocation of photosynthates from shoots where carbon supply exceeds sink demands to inflorescences on proximal shoots where LA is curtailed and insufficient to supply the fertilization process. In this experiment, vines subjected to severe leaf removal (10 basal leaves) in target shoots led to a significant decrease in fruit set when nontarget shoots were limited in their ability to translocate assimilates (UT-LR), whereas defoliated target shoots adjacent to nontarget shoots with a high capacity for translocation (TFR-LR) produced a fruit set similar to untreated vines. Additionally, untreated target shoots did alter fruit set from that of untreated vines when excess assimilates were hypothetically available from nontarget shoots (TFR-UT). This implies that fruit set is dependent upon the whole canopy source/sink balance instead of the retained, available LA within individual shoots. Knowledge of this phenomenon is of special interest to better comprehend the effects of early leaf removal from a practical viewpoint, since this technique is extensively used in cool-climate viticultural regions of the midwestern US. Fruit-set reduction should be more uniform between shoots than the source/sink balance within individual shoots, which can vary greatly in response to manual and mechanical leaf-removal applications.

Literature Cited

Acimovic D, Tozzini L, Green A, Sivilotti P and Sabbatini P. 2016. Identification of a defoliation severity threshold for changing fruit-set, bunch morphology and fruit composition in Pinot noir. Aust J Grape Wine Res 22:399-408.

Baskerville GL and Emin P. 1969. Rapid estimation of heat accumulation from maximum and minimum temperatures. Ecology 50:514-517.

Bennett J, Jarvis P, Creasy GL and Trought MCT. 2005. Influence of defoliation on overwintering carbohydrate reserves, return bloom, and yield of mature Chardonnay grapevines. Am J Enol Vitic 56:386-393.

Candolfi-Vasconcelos M and Koblet W. 1990. Yield, fruit-quality, bud fertility and starch reserves of the wood as a function of leaf removal in *Vitis Vinifera* – evidence of compensation and stress recovering. Vitis 29:199-221.

Candolfi-Vasconcelos MC, Candolfi MP and Koblet W. 1994. Retranslocation of carbon reserves from the woody storage tissues into the fruit as a response to defoliation stress during the ripening period in *Vitis vinifera* L. Planta 192:567-573.

Caspari HW, Lang A and Alspach P. 1998. Effects of girdling and leaf removal on fruit set and vegetative growth in grape. Am J Enol Vitic 49:359-366.

Eichhorn KW and Lorenz DH. 1977. Phänologische Enwicklungsstadien der Rebe. Nachrbl Dtsch Pflanzenschutzd (Braunschweig) 29:119-120.

Flore JA and Lakso AN. 1989. Environmental and physiological regulation of photosynthesis in fruit crops. Hortic Rev 11:111-157.

Frioni T, Acimovic D, Tombesi S, Sivilotti P, Palliotti A, Poni S and Sabbatini P. 2018. Changes in within-shoot carbon partitioning in Pinot noir grapevines subjected to early basal leaf removal. Front Plant Sci 9:1122.

Gatti M, Bernizzoni F, Civardi S and Poni S. 2012. Effects of cluster thinning and preflowering leaf removal on growth and grape composition in cv. Sangiovese. Am J Enol Vitic 63:325-332.

Gatti M, Pirez FJ, Frioni T, Squeri C and Poni S. 2018. Calibrated, delayed-cane winter pruning controls yield and significantly postpones berry ripening parameters in *Vitis vinifera* L. cv. Pinot noir. Aust J Grape Wine Res 24:305-316.

Hale CR and Weaver RJ. 1962. The effect of developmental stage on direction of translocation of photosynthate in *Vitis vinifera*. Hilgardia 33:89-131.

Hed B and Centinari M. 2018. Hand and mechanical fruit-zone leaf removal at prebloom and fruit-set was more effective in reducing crop yield than reducing bunch rot in 'Riesling' grapevines. HortTechnology 28:296-303.

Hunter JJ and Visser JH. 1988. Distribution of ^{14}C-photosynthetate in the shoot of *Vitis vinifera* L. cv Cabernet Sauvignon. I. The effect of leaf position and developmental stage of the vine. S Afr J Enol Vitic 9:3-9.

Hunter JJ and Visser JH. 1990. The effect of partial defoliation on growth characteristics of *Vitis vinifera* L. cv. Cabernet Sauvignon I. Vegetative growth. S Afr J Enol Vitic 11:18-25.

Intrigliolo DS, Lakso AN and Centinari M. 2009. Effects of the whole vine versus single shoot-crop level on fruit growth in *Vitis labruscana* 'Concord.' Vitis 48:1-5.

Keller M. 2015. The Science of Grapevines: Anatomy and Physiology. Academic Press.

Kliewer WM and Dokoozlian NK. 2005. Leaf area/crop weight ratios of grapevines: Influence on fruit composition and wine quality. Am J Enol Vitic 56:170-181.

Lakso AN and Sacks GL. 2009. Vine balance: What is it and how does it change over the season? *In* Proceedings of Recent Advances in Grapevine Canopy Management, A Symposium Dedicated to Emeritus Prof. W. Mark Kliewer. pp. 21-26. University of California, Davis, CA.

Lampinen BD, Tombesi S, Metcalf SG and DeJong TM. 2011. Spur behaviour in almond trees: Relationships between previous year spur leaf area, fruit bearing and mortality. Tree Physiol 31:700-706.

Lebon G, Wojnarowiez G, Holzapfel B, Fontaine F, Vaillant-Gaveau N and Clément C. 2008. Sugars and flowering in the grapevine (*Vitis vinifera* L.). J Exp Bot 59:2565-2578.

Lemoine R et al. 2013. Source-to-sink transport of sugar and regulation by environmental factors. Front Plant Sci 4:272.

Mansfield TK and Howell GS. 1981. Response of soluble solids accumulation, fruitfulness, cold resistance, and onset of bud growth to differential defoliation stress at véraison in Concord grapevines. Am J Enol Vitic 32:200-205.

Marsal J, Basile B, Solari L and Dejong TM. 2003. Influence of branch autonomy on fruit, scaffold, trunk and root growth during stage III of peach fruit development. Tree Physiol 23:313-323.

May P. 2004. Flowering and Fruitset in Grapevines. Lythrum Press, Adelaide, Australia.

Obeso JR. 2002. The costs of reproduction in plants. New Phytol 155:321-348.

Palliotti A, Gatti M and Poni S. 2011. Early leaf removal to improve vineyard efficiency: Gas exchange, source-to-sink balance, and reserve storage responses. Am J Enol Vitic 62:219-228.

Poni S and Bernizzoni F. 2010. A three-year survey on the impact of pre-flowering leaf removal on berry growth components and grape composition in cv. Barbera vines. J Int Sci Vigne Vin 44:21-30.

Poni S, Bernizzoni F, Briola G and Cenni A. 2005. Effects of early leaf removal on cluster morphology, shoot efficiency and grape quality in two *Vitis vinifera* cultivars. Acta Hortic 689:217-226.

Poni S, Casalini L, Bernizzoni F, Civardi S and Intrieri C. 2006. Effects of early defoliation on shoot photosynthesis, yield components, and grape composition. Am J Enol Vitic 57:397-407.

Poni S, Bernizzoni F and Civardi S. 2008. The effect of early leaf removal on whole-canopy gas exchange and vine performance of *Vitis vinifera* L. 'Sangiovese.' Vitis 47:1-6.

Quinlan JD and Weaver RJ. 1970. Modification of pattern of photosynthate movement within and between shoots of *Vitis vinifera* L. Plant Physiol 46:527-530.

Reyes F, DeJong T, Franceschi P, Tagliavini M and Gianelle D. 2016. Maximal growth potential and periods of resources limitation in apple tree. Front Plant Sci 7:233.

Scholefield PB, Neales TF and May P. 1978. Carbon balance of the Sultana vine (*Vitis vinifera* L.) and the effects of autumn defoliation by harvest-pruning. Aust J Plant Physiol 5:561-570.

Sokal RR and Rohlf FJ. 1969. Biometry. The Principles and Practices of Statistics in Biological Research. 2nd ed. W.H. Freeman, San Francisco.

Tombesi S, Lampinen BD, Metcalf S and DeJong TM. 2015. Spur fruit set is negatively related with current-year spur leaf area in almond. Hortscience 50:322-325.

VanderWeide J, Medina-Meza IG, Frioni T, Sivilotti P, Falchi R and Sabbatini P. 2018. Enhancement of fruit technological maturity and alteration of the flavonoid metabolomic profile in Merlot (*Vitis vinifera* L.) by early mechanical leaf removal. J Agric Food Chem 66:9839-9849.

Zapata C, Deléens E, Chaillou S and Magné C. 2004. Partitioning and mobilization of starch and N reserves in grapevine (*Vitis vinifera* L.). J Plant Physiol 161:1031-1040.

Zimmerman MH. 1971. Storage, mobilization and circulation of assimilates. *In* Trees, Structure and Function. Zimmermann MH and Brown CL (eds.), pp. 307-319. Springer, Berlin, Heidelberg, New York.